generate molecules of core structure M, which have a plurality of moieties, each of which can be individually deprotected or subsequently derivatized are provided. In one process, M is a multifunctional low molecular weight compound, such as a saccharide, aminosugar, deoxysugar, nucleoside, nucleotide, coenzyme, amino acid, lipid, steroid, vitamin, hormone, alkaloid, or small molecule drug. In another process, M is an oligomeric compound, such as an oligosaccharide, oligonucleotide, peptide or protein.—

IN THE SPECIFICATION:

Please amend the specification as follows (a marked-up copy of the amended specification is attached to this Amendment):

Please replace the paragraph beginning on page 1, line 25, through the paragraph on page 2, line 7, with the following paragraph:

H-phosphonate or phosphoramidite chemistries employing solid phase methods in automated DNA synthesizers are most efficient for the synthesis of oligonucleotides. The phosphoramidite method using B-cyanoethyl phosphoramidites as reactive nucleotide building blocks is the most prevalent synthesis method due to the quantitative condensation yields despite an oxidation step in every cycle (Sinha, N.D. *et al., Tetrahedron Lett.,* 1983, 24, 5843-46; Sinha, N.D. et al., Nucleic Acids *Res.,* 1984, 125 4539-57; Froehler, B.C. et al., *Nucleic Acids* Res., 1984, 14, 5399-5407; Froehler, B.C. and Matteuci, M.D., *Tetrahedron Lett.*, 1986, 27, 469-72; Garegg, P.J. *et al., Tetrahedron Lett.,* 1986, 27, 4051-54; Sonveaux, E., *Bioorg. Chem,*, 1986, 14, 274-325; Uhimann, E. and Peyman, A., *Chem. Rev.*, 1990, 90, 543-84).

Please replace the paragraph on page 9, lines 3-14, with the following paragraph:

As shown in scheme 1, a 2'-deoxyoligonucleotide, **3**, is synthesized, e.g. by the phosphoramidite method (Sinha, N.D., Biernat, J., Köster, H. *Tetrahedron Lett.,* **1983,** 24, 584346; Sinha, N.D., Biernat, J., McManus, J., Köster, H.

Nucleic Acids Res., **1984,** 12, 4539-57; Sonveaux, E. *Bioorg. Chem.,* **1986,** 14, 274-325). However, in contrast to the usual 3' to 5' addition, the synthesis is performed in the 5' to 3' direction using the building blocks **1** and **2.** During an elongation cycle, the temporary protecting group, R^3, is removed, e.g. using a neutral hydrazine reagent IV (table 1) before the condensation step and the acidified filtrate of the hydrazinolysis solution is spectrophotometrically measured to determine the preceding condensation yield. In this manner, a trityl assay as typically used with the 4, 4'-dimethoxytrityl group, is possible. In addition, there is little risk of depurination, since acidic conditions are not used during the synthesis cycles.

Please replace the paragraph beginning on page 11, line 8, through page 12, line 2, with the following paragraph:

The selective and orthogonal deprotections and the derivatizations by introducing new substituents can be carried out at positions ①-④, at ② and ③, in a *sequence specific* way. During the derivatizations at ①-④ only the npeoc/npe base protection remains intact. In contrast, the phosphate protecting group R^{4B} needs to remain intact if derivatizations at ② are to be performed. These two protecting groups only serve to carry out *sequence specific* derivatizations at ② and/or ③. *After* the derivatizations at least the bases, protected with npeoc/npe groups have to be deprotected without removing new substituents at ①-④ at the same time. The removal of the npeoc/npe groups is necessary to guarantee sufficient hybridization properties of the derivatized oligomers with complementary nucleic acid sequences.

Please replace the paragraph on page 14, lines 14-19, with the following paragraph:

Compared to current oligodeoxynucleotide syntheses for use in antisense and triplex DNA therapies (Cohen, J.S., Hogan, M.E., *Scientific American, Int. Ed.*, December **1994**, pages 50-5514; Uhlmann, E., Peyman, A., *Chem Rev.*, **1990**, 90, 543-84; Beaucage, S.L., Iyer, r.P. Tetrahedron, **1993,** 49, 6123-94),

the new strategy shows a remarkable advantage. All possible derivatizations can be performed with only *one* oligonucleotide synthesis run.

Please replace the paragraph beginning on page 14, line 20, through page 15, line 10, with the following paragraph:

The strategy presented above, can be modified according to other oligonucleotide synthesis schemes. For example, in addition to the phosphoramidite method shown in scheme 1, the strategy can be employed with the phosphotriester and other suitable methods of oligonucleotide synthesis. For the phosphotriester method, chloro substituted phenyl groups and the ß-cyanoethyl group were successfully used as phosphate protection groups (Amarnath, V., Broom, A. D. *Chem. Rev.* **1977**, 77, 183-217; Reese, C. B., *Tetrahedron,* **1978**, 34, 3143-79). The levulinic acid ester and the npeoc/npe base protection are stable during the reaction conditions of the phosphotriester method (Himmelsbach, F., Schulz, B.S., Trichtinger, T., Ramamurthy, C., Pfleiderer, W., *Tetrahedron,* **1984**, 40, 59-72; van Boom, J.H., Burgers, P.M.J., *Tetrahedron Lett.*, **1976**, 4875-78). The nps base protection has been successfully used during the oligonucleotide synthesis by the phosphotriester approach (Heikkila, J., Balgobin, N., Chattopadhyaya. J., *Acad Chem. Sci.*, **1983**, B37, 857-62). The structure of oligomers obtained in this way of synthesis is the same as for the oligomer **3** generated by the phosphoramidite method (scheme 1). For syntheses by the phosphoramidite method, amidites, whose 5'-OH or 3'-OH groups respectively are protected with the 4,4'-dimethoxytrityl (DMTR) group, are used. Scheme shows a general view and scheme 4 to 6 show specific examples.

Please replace the paragraph on page 26, lines 4-14, with the folloing paragraphs:

a: protection of compound **42** with **39** (scheme 8), **32** (scheme 7)[1]),),4-dinitro- or 2-nitrophenylsulfenyl chloride b: aminolysis with aminopropyl CPG, followed by reaction with Fmoc chloride c: orthogonal deprotections of the

Fmoc (9-fluorenylmethoxycarbonyl) group and group R^6 and derivatizations with new substituents R^8 and R^9 respectively, orthogonal deprotection of the levulinic ester moiety d: reaction with succinic anhydride e: reaction of compound **46** with **47**. Compound **47** is (as compound **46**) a derivative of compound **44** but *otherwise derivatized* (with new substituents R^{10} and R^{11} in compound **47** in contrast to R^8 and R^9 in **46**). **47** was removed from the support. The reaction to **48** can be carried out as described by Gupta, K.C. *et al.*, *Nucl. Acids Res.*, **1991**, 19, 3019-25. The successful reaction to **48** can be monitored by vis spectroscopy of **48** after treatment with acid.

Please replace the paragraph on page 27, lines 4-23, with the following paragraph:

The structure of the protecting groups is very useful for the reaction control by thin layer chromatography (tlc) during the synthesis of compound **43**. Each reaction step can be controlled by a specific colorimetric effect and UV-detection. This is demonstrated by the following description. If a compound **42** with e.g. four hydroxyl groups is monosubstituted by compound **32** (scheme 7), treatment with acid leads to an orange product (trityl cation), but the colorimetric trityl moiety is not cleaved. After the second monosubstitution with **39** (scheme 8), detection with acid leads to two orange products, because one of the trityl moieties is now cleaved off. Additionally, intensive yellow color can be observed by ammonia vapour (or by primary and secondary amines), due to released p-nitrophenolate ions. The product obtained after the third monosubstitution with dnps chloride already shows yellow color without any detection reagent (and of course the other colorimetric effects). Protection of the last free hydroxyl group with Fmoc chloride should be done *after* the reaction of compound **43** with aminopropyl CPG, because of the sensitivity of Fmoc esters in the presence of amino groups. Nevertheless, the last free hydroxyl group of a sample of compound **43** can be substituted by a nucleoside derivative (the reactive form of 5'-*O*-DMTr-T_d-*O*3'-succinic mono ester e.g.). By

contact with sugar spray reagent and heating with a fan an *additional* green colored product can be observed on tlc (due to the superposition of the blue color of the nucleoside and the orange color of the trityl moieties). This shows the possibility to control four successive monosubstitions by different colorimetric effects.

Please replace the paragraph beginning on page 28, line 16, through the paragraph on page 29, line 24, with the following paragraph:

^{1}H (400 and 250 MHz) and ^{13}C)101 and 63 MHz) NMR spectra were recorded on a Bruker AMX 400 and a AC 250-P instrument. Samples were dissolved in the presence of tetramethylsilane as internal standard, unless otherwise stated. ^{31}P NMR spectra were recodered on a Varian Gemini 200 instrument. Internal standard: phosphoric acid in the solvent used for the sample (δ = 0.00 ppm), Chemical shifts are given in ppm. Mass spectra were obtained on a Finnigan MAT 311A mass spectrometer under EI conditions, a VG Analytical 70-250S mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol, Xenon bombardment) and a Finnigan MAT Vision 2000 mass spectrometer under MALDI-TOF conditions (matrix solution: 0.7 mol/ 13-hydroxy picolinic acid and 0.07 mol/ 1 ammonium citrate in acetonitrile/ water, 1/1, v/v). Elementary analyses were performed by the analytical department of the Institute of Organic Chemistry, University of Hamburg. Thin layer chromatography (tlc) was carried out on 60 PF_{254} silica gel coated alumina sheets (Merck, Darmstadt, No 5562). Trityl and sugar containing compounds are visualized with sugar spray reagent (0.5 ml 4-methoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a fan or on a hot plate. p-Nitrophenyl ester containing compounds are visualized by ammonia vapour. Column chromatography was performed using silica gel from Merck. HPLC results were obtained on a Waters chromatography systems 625 LC with a photodiodearray detector 996 and using reversed phase columns (Waters Nova-Pak C18, 60 Å, 4 μm particles, 3.9

x 300mm, software: Millenium 2.0, eluants were: 0.1 M triethylammonium acetate at pH 7.0 (A) and acetonitrile (B); the column was equilibrated at 30°C at 1ml per min, with 95% A/ 5% B, v/v, with elution using a linear gradient from 5% to 40% B in 40 min, monitored at 254 nm). Spectrophotometric measurements in the UV/ Vis region were performed on a Beckman UV35 and a LKB Ultrospec Plus UV/ Vis spectrophotometer. Solvents were dried and purified before use according to standard procedures. Extractions were monitored by tlc to optimize completion of extraction.

Please replace the paragraph on page 30, lines 2-24, with the following paragraph:

Compound **32** was prepared *in situ* by reacting levulinic acid derivate **31** (Leikauf, E., Köster. H., *Tetrahedron,* **1995,** 51, 5557-62) (3.78 g, 8.39 mmol) with N,N'dicyclohexylcarbodiimide (1.80 g, 8.74 mmol) in dry dioxane (25 ml). N,N'-dicyclohexylurea is removed by filtration and washed with dioxane. The solution was divided in four equal parts and the solvents were evaporated *in vacuo.* To each of the four residues of anhydride **32** was added one of the four following protected nucleosides: 5'-*O*-DMTr-2'-deoxythymidine, 5'-*O*-DMTr-N^4-npeoc-2'-deoxycytidine, 5'-*O*-DMTr-N^6-npeoc-2'-deoxyadenosine, 5'-*O*-DMTr-N^2-npeoc-O^6npe-2'-deoxyguanosine (1.00 mmol of each; base protected deoxynucleosides were from Chemogen, Konstanz) (Stengele, K.P., Pfleiderer, W., *Tetrahedron* Lett., **1990,** 31, 2549-52) and 4-dimethylaminopyridine (0.0100 g, 0.0819 mmol) in 1.64 ml pyridine. Completion of reaction was checked by thin layer chromatography. 30 min after the addition of a mixture of 0.130 ml of glacial acetic acid and 0.245 ml pyridine, 0.046 ml water were added, 60 min later an excess of ethyl acetate was added, the N,N'-dicyclohexylurea removed by filtration and washed with ethyl acetate. The mixture was extracted with water, 5% aqueous sodium hydrogen carbonate and water. After drying with sodium sulfate, the solvent was evaporated, then co-evaporated with toluene. The residues were directly detritylated with 80%

acetic acid and the reaction was monitored by thin layer chromatography. The solutions were poured into an excess of water (about 10 fold) and the aqueous mixtures were extracted with ethyl acetate. The organic phase was washed with 5% aqueous sodium hydrogen carbonate and water. After drying, the solvent was evaporated, then co-evaporated with toluene (to remove remaining acetic acid). The residues were directly methylated by adding to each a solution of 200 ml methanol and 1 ml glacial acetic acid. If there were some insoluble material, it was dissolved in 5-10 ml dichloromethane and a mixture of 100 ml methanol and 0.5 ml glacial acetic acid was added. Monitoring by thin layer chromatography indicates completion of the reaction. The solvents were evaporated under reduced pressure, followed by co-evaporation with toluene (2-3 times). The residues of **30a-d** were purified by silica gel column chromatography (**30a**: silica gel 60H, No. 7736, **30b-d**: silica gel 60, No. 9385; Merck, Darmstadt). Silica gel used per gram raw product: **30a**: 25 g, **30b**: 51 g, **30c**: 65 g, **30d**: 51 g; using a step gradient from dichloromethane to dichloromethane/ methanol 98/2 (v/v), in the presence of 0.1% pyridine. Pure fractions were pooled, the solvents removed by evaporation, the residues dissolved in dichloromethane (15 ml per gram residue) and the solutions precipitated into hexane (315 ml per gram residue). Yields: **30a**: 68%, **30b**: 63%, **30c**: 62%, **30d**: 52%.

Please replace the paragraph beginning on page 34, line 24, through the paragrapg on page 35, line 22, with the following paragraph:

All steps were carried out under inert atmosphere (argon). Organic solvents were free from water and other impurities. Compounds **30a-d** (0.5 mmol of each) were azeotropically dried with small amounts of pyridine and toluene and dissolved in 2.43 ml ethyl acetate. After the addition of N,N-diisopropyl ethylamine (1.75 mmol, 0.226 g, corresponding to 0.30 ml at room temperature) the reaction flask was capped with a septum and cooled with an ice bath. Chloro-β-cyanoethoxy-N,N-diisopropylaminophosphane (0.610 mmol,

0.144 g, corresponding to 0.117 ml at room temperature, Biosyntech, Hamburg) was added dropwise by a syringe. 15 min later the reaction was allowed to raise to room temperature. Monitoring by thin layer chromatography (about 60 min after starting the reaction) indicated complete conversions to the amidites **2a-d**. The precipitated amine hydrochloride was filtered off using a column type reactor fitted with a sintered glass fritt and washed with 1.5 ml ethyl acetate. The solution was extracted in a separation funnel with cold 5% sodium hydrogen carbonate (2 x 2.8 ml). The organic solution was filtered using the described reactor which contains sodium sulfate, followed by washing of the sodium sulfate layer with ethyl acetate (2 x 1.8 ml). After evaporation of the solvents of the filtrate, a foam was obtained. The amidite was dissolved in 5 ml ethyl acetate (containing 0.1% pyridine) and precipitated into 120 ml of hexane (at -20°C). After filtration using the described reactor the amidite was washed with 12 ml of hexane, dried and stored at 20°C. Yields: **2a**: 86%, 2b: 72%, 2c: 78%, 2d: 80%. - ^{31} *P NMR* (81 MHz, CD_3CN/ CH_3CN, I/I,v/v and a trace of N,N-diisopropyl ethylamine): **2a**: δ = 149.18, 149.35 (diastereomers), **2b**: δ = 149.25, **2c**: δ = 149.07, **2d**: δ = 148.89, 149.16 (diastereomers).

Please replace the paragraph on page 36, lines 4-29, with the following paragraph:

Compound 35 (25.7 g, 144 mmol) and methoxybenzene (36.8 g, 340 mmol) were stirred in 450 ml glacial acetic acid to dissolve most of the material. The mixture was cooled in an ice bath and immediately concentrated sulfuric acid (225 g, 2290 mmol) added dropwise. The reaction mixture was then stirred at room temperature until thin layer chromatography (dichloromethane/methanol: 8/2, v/v) demonstrated quantitative conversion. The reaction mixture was poured into 3/1 ice/ water. Subsequently the reaction flask was washed with ether and the ether solution was poured into the ice/water. The orange-white raw product between the aqueous and organic layer was filtrated by suction (if there was still a considerable amount of the raw

product under the aqueous and/or dissolved in the ether layer, it was also worked up). The raw product was triturated with 200 ml water, filtrated by suction, again triturated with petroleum ether (bp 60-70° C) and filtrated. It was recrystallized from ether. Yield: 22.3 g (41%). Note: More product **36** can be purified from the crystalline residue of the mother liquor by silica gel column chromatography or by Soxhlet extraction with petroleum ether (bp 30-50°C). *^{1}H NMR* (250 MHz, $CDCl_3$): δ = 2.65 (t, 2H, -**C**H_2-CH_2-), 2.92 (t, 2H, -CH_2--**C**H_2-),3.78 (s, 6H, -O**C**H_3), 5.40 (s, 1H, R_3C-**H**), 7.13-6.77 (m, 12H, aryl-**H**). - *^{13}C NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm): δ = 30.1 (t, -**C**H_2-CH_2-), 35.48 (t, -CH_2-**C**H_2-), 54.8, 55.19 (q, aryl-O**C**H_3 and d, R_3**C**-H, position not defined), 113.63, 128.1, 129.41, 130.21 (d, **C**H, aryl), 136.46, 137.91, 142.7 (s, aryl, quartenary), 157.93 (s, R_2**C**-OCH_3, aryl), 178.85 (s, --**C**OOH).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (100), 345 (9, M-OCH_3 $^+$), 227 (35, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (48), 345 (8, M-OCH_3 $^+$), 269 (53, M - C_6H_4-OCH_3 $^+$), 227 (38, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *Elementary Analysis* (%): Found: C, 76.55/ 76.35; H, 6.71/6.53;$C_{24}H_{24}O_4$ requires C, 76.57; H, 6.43.

Please replace the paragraph beginning on page 40, line 22, through the paragraph on page 41, line 7, with the following paragraph:

Compound **40** (0.160 g, 0.135 mmol) was dissolved in dry dioxane (0.311 ml) and dry pyridine (0.032 ml). A suspension of aminopropyl CPG (0.405 g, CPG-10-500, Biosyntech, Hamburg) in 1.27 ml dry N,N-dimethylformamide and 0.160 ml (0.116 g, 1.15 mmol) dry triethylamine was added and the suspension shaken during 21.5 h. An intensive yellow color indicated beginning reaction caused by released p-nitrophenolate ions. The suspension was shaken during 21.5 h. A ninhydrin test at this stage indicated the existence of free amino groups on the support. To acylate, "cap", these groups, dry triethylamine (0.030 ml) and acetic anhydride (0.090 ml) were

added and the suspension was shaken for another 60 min. After this time a negative ninhydrin test was obtained. The support was washed successively with N,N-dimethylformamide, ethanol, dioxane, ether (100 ml each) and dried *in vacuo.* Analysis for the extent of 3'-OH protected nucleoside attached to the support was done spectrophotometrically. An accurately weighed sample was treated either with 5% dichloroacetic acid in dichloromethane (v/v) or with hydrazine reagent IV (table 1) followed by acidifying the solution with 40% trichloroacetic acid in dichloromethane (percentage by weight). The liquid phase was measured at 513 nm (extinction coefficient of an acid solution of the removed trityl derivate: ε = 78600). Amount of nucleoside bound to the support **1**:45.6 μmol/g.

Please replace the paragraph on page 42, lines 8-11, with the following paragraph:

6) *Hydrazine reagent*: 0.5M hydrazine reagent IVb (table 1). Reagent of high quality have to be used: bidistilled water, acetic acid p.a. (Merck, Darmstadt No. 63), hydrazinium hydrate (Merck, Darmstadt No. 804608), pyridine p.a. (Merck, Darmstadt No. 7463).

Please replace the paragraph on page 44, lines 4-12, with the following paragraph:

The support with the attached oligomer was washed with pyridine and the β-cyanoethyl groups were removed with *tert*-butyl amine reagent II (table 1). After washing the support with pyridine and acetonitrile and drying *in vacuo,* the oligomer was removed from the support by treating it with 80% acetic acid for 15 min. After lyophilization of the solution, the oligomer was purified by HPLC: the terminal 3'-OH protecting group (corresponding to the group of compound **29a** in scheme 7) served here as purification handle. Treatment with 32% ammonia followed by lyophilisation led to the fully deprotected oligomer d(TTTT). - *HPLC:* Ret. time (min): 8.57, UV detection: λmax = 266.1 and 217.7 nm. - MS (MALDI-TOF): theoretical mass: M+H +: 1155; found: 1154.